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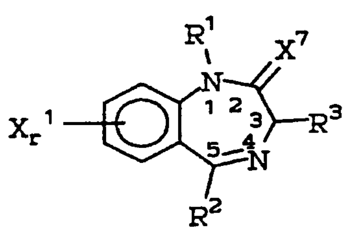
(54) **New benzodiazepine analogs.**

(57) Pharmaceutical compositions containing benzodiazepine analogs of the formula :



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are disclosed which are antagonists of gastrin and cholecystokinin (CCK) with enhanced aqueous solubility and have properties useful in the treatment of oncologic disorders, controlling pupil constriction in the eye, or treating a withdrawal response produced by treatment or abuse of drugs or alcohol.

EP 0 523 845 A2

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CROSS-REFERENCE

Starting materials for the compounds of Formula I are prepared and described in U.S. Patent 4,820,834 and B. Evans et al., J. Med. Chem. 31, 2235-2246 (1988), both incorporated by reference for these purposes.

BACKGROUND OF THE INVENTION

Cholecystokinins (CCK) and gastrin are structurally-related neuropeptides which exist in gastrointestinal tissue and in the central nervous system (see, V. Mutt, Gastrointestinal Hormones, G B. J. Glass, Ed., Raven Press, N.Y., p. 169 and G. Nisson, ibid, p. 127).

The isolation of the 33-amino acid polypeptide, cholecystokinin (CCK-33), from porcine intestine, Mutt, V. et al., "Structure of Porcine Cholecystokininpancreozymin. 1. Cleavage with Thrombin and Trypsin", European J. Biochem. 6, 156, (1968), was followed by the discovery that it occurs in numerous molecular forms at various sites throughout the peripheral and central nervous systems, Larsson, L. et al., "Localization and Molecular Heterogeneity of Cholecystokinin in the Central and Peripheral Nervous System", Brain Res., 165, 201 (1979). In the mammalian brain the predominant fragments are the carboxy terminal octapeptide, H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (CCK-8s, CCK_{26-33}) and tetrapeptide, CCK-4 (CCK_{30-33}).

The carboxy terminal octapeptide possesses the full biological profile of CCK, Dockray, G.J. et al., "Isolation, Structure and Biological Activity of Two Cholecystokinin Octapeptides from Sheep Brain", Nature 274, 711 (1978), and meets many anatomical and biochemical criteria which characterize a neurotransmitter, Vanderhaeghen, J.J. et al., "J. Neuronal Cholecystokin in", Ann. N.Y. Acad. Sci., 448, (1985). The presence of high concentrations of CCK-8s in the mammalian CNS is complemented with findings of specific and high affinity membrane-bound CCK binding sites, Innis, R.B. et al., "Distinct Cholecystokinin Receptors in Brain and Pancreas", Proc. Natl. Acad. Sci. U.S.A., 77, 6917 (1980).

Evidence that more than one form of CCK receptor might exist was first provided in 1980 by Innis and Snyder, Innis, R.B. et al., "Distinct Cholecystokinin Receptors in Brain and Pancreas", Proc. Natl. Acad. Sci. U.S.A., 77, 6917 (1980). At present, CCK receptors have been differentiated into primarily two subtypes based on their affinity for CCK fragments and analogues, Innis, R.B. et al., "Distinct Cholecystokinin Receptors in Brain and pancreas", Proc. Natl. Acad. Sci. U.S.A., 77, 6917 (1980). The subsequent development of agents which discriminate between different CCK receptor types afforded further support for these assignments, Chang, R.S.L. et al., "Biochemical and Pharmacological Characterization of an Extremely Potent and Selective Nonpeptide Cholecystokinin Antagonist", Proc. Natl. Acad. Sci. U.S.A., 83, 4923 (1986).

The CCK-A receptors, previously known as peripheral CCK receptors, are located in organs such as the pancreas, gallbladder, and colon. They exhibit high affinity for CCK-8s and a lower affinity for the corresponding desulphated fragment, CCK-8d, for CCK-4, and gastrin. Recent autoradiographic results have localized CCK-A receptors in the brain as well, Hill, D.R. et al., "Autoradiographic Localization and Biochemical Characterization of Peripheral Type CCK Receptors in Rat CNS Using Highly Selective Nonpeptide CCK Antagonists", J. Neurosci., 7, 2967 (1987).

The majority of the CCK receptors in the brain are of the CCK-B type. These were previously designated as central CCK receptors. CCK-B receptors are widely distributed throughout the brain and display high affinity for CCK-8s, CCK-4, and pentagastrin, Hill, D.R. et al., "Autoradiographic Localization and Biochemical Characterization of Peripheral Type CCK Receptors in Rat CNS Using Highly Selective Nonpeptide CCK Antagonists", J. Neurosci, 7, 2967 (1987).

In addition to the above mentioned CCK receptor subtypes is a third type, the stomach gastrin receptor, which appears to be closely related to the CCK-B receptor subtype, Beinfeld, M.C., "Cholecystokinin in the Central Nervous System; a Minireview", Neuropeptides, 3, 4111 (1983). The minimum fully potent CCK sequence at this receptor is CCK-4, Gregory, R.A., "A Review of some Recent Development in the Chemistry of the Gastrins", Biorg. Chem., 8,497 (1979).

A wide range of physiological responses has been attributed to CCK. In an effort to elucidate its biological roles, researchers have relied primarily on a collection of CCK-A antagonists which has been steadily supplemented and improved to now include very selective, high-affinity agents, Evans, B.E., "Recent Developments in Cholecystokinin Antagonist Research," Drugs Future, 14, 971 (1989). In addition to their value as investigative tools, CCK antagonists retain considerable therapeutic potential, Gertz, B.J., "Potential Clinical Applications, of a CCK Antagonist in Cholecystokinin Antagonists," Alan R. Liss, Inc.: New York, pp. 327 (1988).

In recent years, interest in agonists and antagonists of CCK has been stimulated by the possible clinical application of such compounds, Silverman, M.A. et al., "Cholecystokinin Receptor Antagonists, a Review", Am. J. Gastroenterol, 82, 703, (1987). The discovery of the presence of CCK in the brain and its significance in

relation to its modulation of dopaminergic functions, effects on satiety, its roles in nociception, in anxiety, and other brain functions, Vanderhaeghen, J.J., et al., "J. Neuronal Cholecystokinin", Ann. N.Y. Acad. Sci. 448 (1985) has understandably intensified the search for CCK-B selective agents. Since the relevant biologically active fragment, CCK-8s, has a half-life of less than 1 hour, Deschodt-Lanckman, K., et al., "Degradation of Cholecystokinin-like Peptides by a Crude Rat Brain Synaptosomal Fraction: a Study by High Pressure Liquid Chromatography", Reg. Pept., 2, 15 (1981), implicit in the development of candidates for clinical use are criteria of high potency, selectivity, long in-vivo duration, oral bioavailability, and capability of penetrating the blood-brain barrier. These are strict prerequisites, given the tenuous stature of peptides as drugs, Veber, D.F., et al., "The Design of Metabolically-stable Peptide Analogs", Trends Neurosci. 8, 392 (1985).

Nevertheless, by employing stratagems which stabilize peptide structures, advances have been made toward developing highly potent and selective peptidal CCK-B receptor ligands Charpentier, B. et al., "Cyclic Cholecystokinin Analogues with High Selectivity for Central Receptors". Proc. Natl. Acad. Sci. U.S.A., 85, 1968, (1988). Analogues are now available which have proven resistant to enzymatic degradation Charpentier, B. et al., "Enzyme-resistant CCK Analogs with High Affinities for Central Receptors", Peptides, 9 835 (1988). Despite favorable receptor binding profiles, this class of compounds fails to meet previously cited key requirements which characterize a drug candidate. In response, researchers have turned to non-peptide compounds which offer a broader range of structure and physicochemical properties.

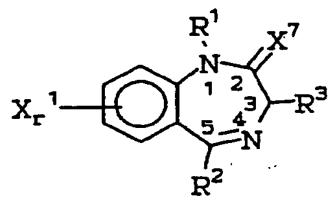
It was, therefore, an object of this invention to identify pharmaceutical compositions containing the compounds of Formula I which are useful in the treatment of oncologic disorders, controlling pupil constriction in the eye, or treating a withdrawal response produced by treatment or abuse of drugs or alcohol.

SUMMARY OF THE INVENTION

It has now been found that pharmaceutical compositions containing compounds of Formula I are useful in the treatment of oncologic disorders, controlling pupil constriction in the eye, or treating a withdrawal response produced by treatment or abuse of drugs or alcohol.

DETAILED DESCRIPTION OF THE INVENTION

The pharmaceutical compositions containing compounds of formula I are represented by the formula:



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wherein:

R^1 is H, C_1-C_6 linear or branched alkyl, loweralkenyl, lower alkynyl, $X^{12}COOH$, $-X^{12}COOR^6$, $-X^{11}$-cycloloweralkyl, $-X^{12}NR^4R^5$, $-X^{12}CONR^4R^5$, $-X^{12}CN$, or $-X^{11}CX_3{}^{10}$;

R^2 is H, loweralkyl, substituted or unsubstituted phenyl (wherein the substitutents may be 1 or 2 of halo, loweralkyl, loweralkoxy, loweralkythio, carboxyl, carboxyloweralkyl, nitro, $-CF_3$, or hydroxy), 2-,3-, or 4-pyridyl;

R^3 is

$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X^{11}R^7,$$

$-NH(CH_2)_{2-3}NHCOR^7$,

$$-X^{11}\overset{O}{\overset{\|}{C}}X^9X^{11}R^7,$$

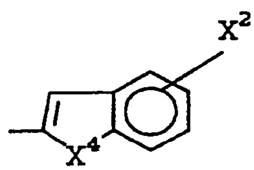
$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X_a^{9}X^{11}R^{7},$$

or $-X^{11}NR^{18}SO_2(CH_2)_qR^7$;

R^4 and R^5 are independently H or R^6 or in combination with the N of the NR^4R^5 group form an unsubstituted or mono or disubstituted, saturated or unsaturated, 4-7 membered heterocyclic ring, or benzofused, 4-7 membered heterocyclic ring wherein said heterocyclic ring or said benzofused heterocyclic ring may contain a second heteroatom selected from O and NCH_3 and the substituent(s) is/are independently selected from C_1-C_4alkyl;

R^6 is loweralkyl, cycloloweralkyl, substituted or unsubstituted phenyl, or substituted or unsubstituted phenylloweralkyl wherein the phenyl or phenylloweralkyl substituents may be 1 or 2 of halo, loweralkyl, loweralkoxy, nitro, or CF_3;

R^7 is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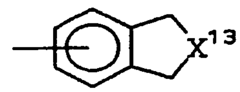
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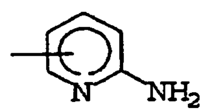
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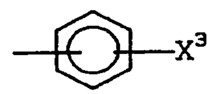
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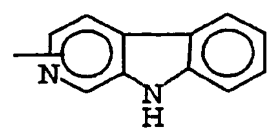
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R^8 is H, loweralkyl, cycloloweralkyl, $-X^{13}CONH_2$, $-X^{13}COOR^6$, $-X^{13}COOH$, $-X^{13}$-cycloloweralkyl, or $-X^{13}NR^4R^5$,

R^{15} is H, loweralkyl, or cycloloweralkyl;

R^{18} is H or loweralkyl;

n is 1-6,

q is 0-4;

r is 1 or 2;

X^1 is H,$-NO_2$,CF_3, CN, OH, loweralkyl, halo, loweralkylthio, loweralkoxy, $-X^{11}COOR^6$, $X^{11}COOH$, or $-X^{11}NR^4R^5$,

X^2 is H or X^3, with the proviso that when X^2 is H, then X^4 is NX^5COOH or NX^5COOR^6 wherein X^5 is a linear alkyl chain of 2 to 6 carbon atoms, any carbon atom of which may be additionally substituted with a linear or branched alkyl group of 1 to 3 carbon atoms;

X^3 is $O(CH_2)_nCOOR^6$, $O(CH_2)_nCOOH$, $(CH_2)_nCOOR^6$, $(CH_2)_nCOOH$, $COOR^6$, or $X^{12}OR^8$;

X^4 is S,O, CH_2, or NR^8;

$X^7$is O,S, HH, or $N(R^{15})_2$ with the proviso that X^7 can be $N(R^{15})_2$ only when R^1 is not H;

X^8 is H, loweralkyl;
X^9 and X_a^9 are independently NR^{18} or O;
X^{10} is F, Cl, or Br;
X^{11} is absent or C_{1-4} linear or branched alkyl;
X^{12} is C_{1-4} linear or branched alkylidene;
X^{13} is C_{1-4} linear or branched alkyl;
or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

One embodiment of the present invention encompasses compounds of formula 1, wherein:

R^1 is H, C_1-C_6 linear or branched alkyl, $-X^{12}COOR^6$, $-X^{11}$-cycloloweralkyl, $X^{12}NR^4R^5$, $-X^{12}CONR^4R^5$, or $X^{12}COOH$;

R^2 is substituted or unsubstituted phenyl (wherein the substitutents may be 1 or 2 of halo, loweralkyl, loweralkoxy, loweralkylthio, carboxyl, carboxyloweralkyl, nitro, $-CF_3$ or hydroxy), 2-,3-, or 4-pyridyl;

R^3 is

$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X^{11}R^7,$$

$$-X^{11}\overset{O}{\overset{\|}{C}}X^9X^{11}R^7,$$

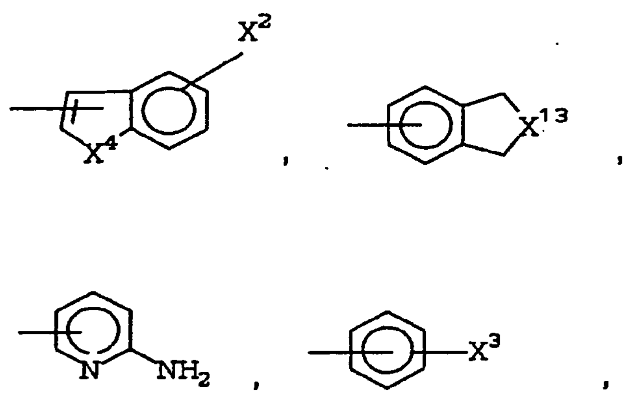
$-NH(CH_2)_{2-3}NHCOR^7$, or

$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X^9X^{11}R^7;$$

R^4 and R^5 are independently H or R^6 or in combination with the N or the NR^4R^5 group form an unsubstituted or mono or disubstituted, saturated or unsaturated, 4-7 membered heterocyclic ring, or benzofused 4-7 membered heterocyclic ring wherein said heterocyclic ring or said benofused heterocyclic ring may contain a second heteroatom selected from O and NCH_3 and the substituent(s) is/are independently selected from C_1-C_4 alkyl;

R^6 is C_{1-4} straight or branched-chain alkyl or C_3-C_6 cycloalkyl;

R^7 is



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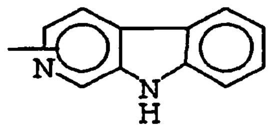


;

R^8 is H, loweralkyl, cycloloweralkyl, $X^{13}COOR^6$, $X^{13}COOH$, or $X^{13}NR^4R^5$;

R^{18} is H or loweralkyl;

n is 1-3;

q is O-3;

r is 1 or 2;

X^1 is H, $-NO_2$, CF_3, CN, loweralkyl, halo, loweralkylthio $-X^{11}COOR^6$, $X^{11}COOH$, or $X^{11}NR^4R^5$;

X^2 is H or X^3 with the proviso that when X^2 is H, then X^4 is NX^5COOH or NX^5COOR^6 wherein X^5 is a linear alkyl chain of 2 to 4 carbon atoms, any carbon atom of which may be additionally substituted with a linear or branched alkyl group of 1 to 3 carbon atoms;

X^3 is $O(CH_2)_nCOOR^6$, $O(CH_2)_nCOOH$, $(CH_2)_nCOOR^6$, $(CH_2)_nCOOH$, or $COOR^6$;

X^4 is S, O, or NR^8;

X^7 is O;

X^9 and X_a^9 are independently NR^{18}, or O;

X^{11} is absent or C_{1-4} linear alkyl;

X^{12} is C_{1-4} linear or branched alkylidene;

X^{13} is C_{1-4} linear or branched alkyl;

or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

As used herein, the definition of each substituent e.g., R^7, loweralkyl, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure. Alkylidene is an alkyl group with two hydrogens abstracted from the same carbon atoms.

As used herein, halo is F, Cl, Br or I; alkyl and loweralkyl are each, unless otherwise indicated, 1-7 carbon straight or branched chain saturated alkyl having one or sometimes two hydrogens abstracted, and includes methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and t-butyl, pentyl, hexyl, and heptyl; in loweralkoxy and loweralkylthio, the alkyl portion is loweralkyl as previously defined; cycloloweralkyl is cycloalkyl of 3-7 carbons; loweralkenyl is 1-5 carbon straight or branched chain alkenyl; acyl is formyl, acetyl, propionyl, benzoyl or butyryl; loweralkynyl is 1-5 carbon straight or branched chain alkynyl.

The pharmaceutically acceptable salts of the compounds of Formulas I include the conventional non-toxic salts or the quarternary ammonium salts of the compounds of Formula I formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the compounds of Formula I which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts are prepared by reacting the free base or acid with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid or base in a suitable solvent or various combinations of solvents.

The pharmaceutically acceptable salts of the acids of Formula I are also readily prepared by conventional procedures such as treating an acid of Formula I with an appropriate amount of a base, such as an alkali or alkaline earth metal hydroxide e.g. sodium, potassium, lithium, calcium, or magnesium, or an organic base such as an amine, e.g., dibenzylethylenediamine, trimethylamine, piperidine, pyrrolidine, benzylamine and the like, or a quaternary ammonium hydroxide such as tetramethylammonium hydroxide and the like.

The compounds of Formula I may further be useful in the treatment of oncologic disorders wherein CCK or gastrin may be involved. Examples of such oncologic disorders include small cell adenocarcinomas and primary tumors of the central nervous system glial and neuronal cells. Examples of such adenocarcinomas and tumors include, but are not limited to, tumors of the lower esophagus, stomach, intestine, colon and lung, including small cell lung carcinoma.

The compounds of Formula I may further be used to control pupil constriction in the eye. The compounds may be used for therapeutic purposes during eye examinations and intraocular surgery in order to prevent mio-

sis. The compounds may further be used to inhibit moisis occurring in association with iritis, uveitis and trauma.

The compounds of Formula I may further be useful for preventing or treating the withdrawal response produced by chronic treatment or abuse of drugs or alcohol. Such drugs include, but are not limited to cocaine, alcohol or nicotine.

The present invention also encompasses a pharmaceutical composition useful in the treatment of CCK and/or gastrin disorders comprising the administration of a therapeutically effective but non-toxic amount of the compounds of Formula I, with or without pharmaceutically acceptable carriers or diluents.

The compounds of Formula I thereof, may be administered to a human subject either alone or, preferably, in combination with pharmaceutically-acceptable carriers or diluents, optionally with known adjuvants, such as alum, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including intravenous, intramuscular, intraperitoneal, subcutaneous and topical administration.

For oral use of an antagonist of CCK, according to this invention, the selected compounds may be administered, for example, in the form of tablets or capsules, or as an aqueous solution or suspension. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch, and lubricating agents, such as magnesium stearate, are commonly added. For oral administration in capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents may be added. For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient are usually prepared, and the pH of the solutions should be suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled in order to render the preparation isotonic.

When a compound according to Formula I is used as an antagonist of CCK or gastrin in a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms. However, in most instances, an effective daily dosage will be in the range of from about 0.005 mg/kg to about 50 mg/kg of body weight, and preferably, of from about 0.05 mg/kg to about 50 mg/kg of body weight, and most preferably, of from about 0.5 mg/kg to about 20 mg/kg of body weight, administered in single or divided doses.

In some cases, however, it may be necessary to use dosage levels outside these limits. For example, doses as low as about 1 ng/kg, about 0.005 μg to about 0.05 μg or about 100 ng to about 100 μg/kg may be administered.

The compounds of Formula I are prepared according to the schemes and descriptions of U.S. Patent 4,820,834 herein incorporated by reference for these purposes. One preferred synthetic scheme is Scheme IVa involving nitrosation, reduction and acylation, according to U.S. Patent 4,820,834. See also Examples 1-5 below.

1. <u>CCK Receptor Binding (Pancreas)</u>

CCK-33 was radiolabeled with ^{125}I-Bolton Hunter reagent (2000 Ci/mmole) as described by Sankara et al. (J. Biol. Chem. <u>254</u>: 9349-9351, 1979). Receptor binding was performed according to Innis and Snyder (Proc. Natl. Acad. Sci. <u>77</u>, 6917-6921, 1980) with the minor modification of adding the additional protease inhibitors, phenylmethane sulfonyl fluoride and o-phenanthroline. The latter two compounds have no effect on the ^{125}I-CCK receptor binding assay.

Male Sprague-Dawley rats (200-350g) were sacrificed by decapitation. The whole pancreas was dissected free of fat tissue and was homogenized in 20 volumes of ice-cold 50 mM, Tris HCl (pH 7.7 at 25°C) with a Brinkmann Polytron PT 10. The homogenates were centrifuged at 48,000 g for 10 min. Pellets were resuspended in Tris Buffer, centrifuged as above and resuspended in 200 volumes of binding assay buffer (50 mM Tris HCl, pH 7.7 at 25°C, 5 mM dithiothrietol, 0.1 mM bacitracin, 1.2 mM phenylmethane sulfonyl fluoride and 0.5 mM o-phenanthroline). For the binding assay, 25 μl of buffer (for total binding) or unlabeled CCK-8 sulfate to give a final concentration of 1 μM (for nonspecific binding) or the compounds of Formula I (for determination of inhibition of ^{125}I-CCK binding) and 25 μl of ^{125}I-CCK-33 (30,000-40,000 cpm) were added to 450 μl of the membrane suspensions in microfuge tubes. All assays were run in duplicate or triplicate. The reaction mixtures were incubated at 37°C for 30 minutes and centrifuged in a Beckman Microfuge (4 minutes) immediately after adding 1 ml of ice-cold incubation buffer. The supernatant was aspirated and discarded, pellets were counted with a Beckman gamma 5000. For Scatchard analysis (<u>Ann. N.Y. Acad. Sci. 51</u>: 660, 1949), ^{125}I-CCK-33 was progressively diluted with increasing concentrations of CCK-33.

2. CCK Receptor Binding (Brain)

CCK-33 was radiolabeled and the binding was performed according to the description for the pancreas method with modifications according to Saito et al., J. Neurochem. 37:483-490, 1981.

Male Hartley guinea pigs (300-500g) were sacrificed by decapitation and the brains were removed and placed in ice-cold 50 mM, Tris HCl plus 7.58 g/l Trizma-7.4 (pH 7.4 at 25°C). Cerebral cortex was dissected and used as a receptor source. Each gram of fresh guinea pig brain tissue was homogenized in 10 ml of Tris/Trizma buffer with a Brinkman polytron PT-10. The homogenates were centrifuged at 42,000 g for 15 minutes. Pellets were resuspended in Tris Buffer, centrifuged as above and resuspended in 200 volumes of binding assay buffer (10 mM N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid (HEPES), 5 mM $MgCl_2$, 0.25 mg/ml bacitracin, 1 mM ethylene glycol-bis-(β-aminoethylether-N,N'-tetraacetic acid) (EGTA), and 0.4% bovine serum albumin (BSA)). For the binding assay, 25 µl of buffer (for total binding) or unlabeled CCK-8 sulfate to give a final concentration of 1µm (for nonspecific binding) or the compounds of Formula I (for determination of inhibition of ^{125}I-CCK binding) and 25 µl of ^{125}I-CCK-33 (30,000-40,000 cpm) were added to 450 µl of the membrane suspensions in microfuge tubes. All assays were run in duplicate or triplicate. The reaction mixtures were incubated at 25°C for 2 hours and centrifuged in a Beckman Microfuge (4 minutes) immediately after adding 1 ml of ice-cold incubation buffer. The supernatant was aspirated and discarded, pellets were counted with a Beckman gamma 5000.

The compounds of Formula I can be determined to be competitive antagonists of CCK according to the following assays.

3. Isolated guinea pig gall bladder

Male Hartley guinea pigs (400-600 g) are sacrificed by decapitation. The whole gall bladder is dissected free from adjacent tissues and cut into two equal halves. The gall bladder strips are suspended along the axis of the bile duct in a 5 ml organ bath under 1 g tension. The organ bath contains a Kreb's bicarbonate solution (NaCl 118 mM, KCl 4.75 mM, CaCl 2.54 mM, KH_2PO_4 1.19 mM, Mg SO_4 1.2 mM, $NaHCO_3$ 25 mM and dextrose 11 mM) maintained at 32°C and bubbled with 95% 0_2 and 5% CO_2. Isometric contractions are recorded using Statham (60 g; 0.12 mm) strain gauges and a Hewlett-Packard (77588) recorder. The tissues are washed every 10 minutes for 1 hour to obtain equilibrium prior to the beginning of the study. CCK-8 is added cumulatively to the baths and EC_{50}'s determined using regression analysis. After washout (every 10 minutes for 1 hour), the compound of Formula I is added at least 5 minutes before the addition of CCk-8 and the EC_{50} of CCK-8 in the presence of the compound of Formula I similarly determined.

4. Isolated longitudinal muscle of guinea pig ileum

Longitudinal muscle strips with attached nerve plexus are prepared as described in Brit. J. Pharmac. 23: ; 356-363, 1964; J. Physiol. 194: 13-33, 1969. Male Hartley guinea pigs are decapitated and the ileum removed (10 cm of the terminal ileum is discarded and the adjacent 20 cm piece used). A piece (10 cm) of the ileum is stretched on a glass pipette. Using a cotton applicator to stroke tangentially away from the mesentery attachment at one end, the longitudinal muscle is separated from the underlying circular muscle. The longitudinal muscle is then tied to a thread and by gently pulling, stripped away from the entire muscle. A piece of approximately 2 cm is suspended in 5 ml organ bath containing Krebs solution and bubbled with 95% O_2 and 5% CO_2 at 37°C under 0.5 g tension. CCK-8 is added cumulatively to the baths and EC_{50} values in the presence and absence of compounds of Formula I determined as described in the gall bladder protocol (above).

5. Gastrin Antagonism

Gastrin antagonist activity of compounds of Formula I is determined using the following assay.

A. Gastrin Receptor Binding in Guinea Pig Gastric Glands

Preparation of guinea pig gastric mucosal glands

Guinea pig gastric mucosal glands were prepared by the procedure of Berglingh and Obrink Acta Physiol. Scand. 96: 150 (1976) with a slight modification according to Praissman et al. C. J. Receptor Res. 3: (1983). Gastric mucosa from guinea pigs (300-500 g body weight, male Hartley) were washed thoroughly and minced with fine scissors in standard buffer consisting of the following: 130 mM NaCl, 12 mM $NaHCO_3$, 3 mM NaH_2PO_4,

3 mM Na_2HPO_4, 3 mM K_2HPO_4, 2 mM $MgSO_4$, 1mM $CaCl_2$, 5 mM glucose and 4 mM L-glutamine, 25 mM HEPES at pH 7.4. The minced tissues were washed and then incubated in a 37°C shaker bath for 40 minutes with the buffer containing 0.1% collagenase and 0.1% BSA and bubbled with 95% O_2 and 5% CO_2. The tissues were passed twice through a 5 ml glass syringe to liberate the gastric glands, and then filtered through 200 mesh nylon. The filtered glands were centrifuged at 270 g for 5 minutes and washed twice by resuspension and centrifugation.

B. Binding studies

The washed guinea pig gastric glands prepared as above were resuspended in 25 ml of standard buffer containing 0.25 mg/ml of bacitracin. For binding studies, to 220 µl of gastric glands in triplicate tubes, 10 µl of buffer (for total binding) or gastrin (1 µM final concentration, for nonspecific binding) or test compound and 10 µl of ^{125}I-gastrin (NEN, 2200 Ci/mmole, 25 pM final) or ^{3}H-pentagastrin (NEN 22 Ci/mmole, 1 nM final) were added. The tubes were aerated with 95% O_2 and 5% CO_2 and capped. The reaction mixtures after incubation at 25°C for 30 minutes were filtered under reduced pressure on glass G/F B filters (Whatman) and immediately washed further with 4 x 4 ml of standard buffer containing 0.1% BSA. The radioactivity on the filters was measured using a Beckman gamma 5500 for ^{125}I-gastrin or liquid scintillation counting for ^{3}H-pentagastrin.

In Vitro Results

Effect of The Compounds of Formula I on ^{125}I-CCK-33 receptor binding

The preferred compounds of Formula I are those which inhibited specific ^{125}I-CCK-33 binding in a concentration dependent manner.

Scatchard analysis of specific ^{125}I-CCK-33 receptor binding in the absence and presence of the compounds of Formula I indicated the compound of Formula I competitively inhibited specific ^{125}I-CCK-33 receptor binding since it increased the K_D (dissociation constant) without affecting the B_{max} (maximum receptor number). A K_i value (dissociation constant of inhibitor) of the compounds of Formula I was estimated.

The data of Table I were obtained for compounds of Formula I.

TABLE I

CCK RECEPTOR BINDING RESULTS IC_{50}(µM)			
Compound of EX#	^{125}I-CCK Pancreas	^{125}I-CCK Brain	^{125}I-Gastrin Gastric Glands
5	0.28	0.002	0.0011
6	0.00013	0.1290	0.07000
7	0.00010	0.2300	0.24000
9	0.04900	0.0039	0.00900
10	0.04900	0.0039	0.00900
12	0.00240	0.1600	0.24000
13	0.01400	0.0710	6.40000
14	2.70000	0.0110	0.40000
15	0.00330	0.9100	
16	0.02300	0.1600	
17	0.06900	0.0120	0.00380
18	2.6	0.024	0.01
19	0.02	0.026	0.018

EXAMPLE 1

1,3-Dihydro-1-methyl-3-oximino-5-phenyl(-2H-1,4-benzodiazepin-2-one

To a suspension of potassium tert-butoxide (24.9 g, 222 mmole) in 600 ml of dry tetrahydrofuran was added 200 ml of dry tert-butylalcohol at -20°C under nitrogen. To this solution was then added via addition funnel 1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (25 g, 99.9 mmole) in 260 ml of tetrahydrofuran. The resulting wine colored solution was stirred for 2 hours at -20°C and treated with 17.4 ml (130 mmole) of isoamyl nitrite. The reaction mixture was warmed to 0°C over 15 minutes and quenched with the addition of 60 ml of cold water and 20 ml of glacial acetic acid. All solvents were removed under reduced pressure and the residue was partitioned between ethyl acetate (600 ml) and brine (100 ml). The phases were separated and the organic extracts were dried (Na_2SO_4) and concentrated. The resulting semi-solid was triturated with ether to give 21 g of off-white solid. m.p. 234-235°C;
R_f=0.15 (ethyl acetate-hexane, 1:1); R_f=0.28 chloroform-ethanol, 95:5);
ir(KBr, partial): 3300, 1650, 1595, 1320, 1205, 1030, 975 cm^{-1}.
MS (14 ev.): 279 (M^+), 262, 249, 236, 222.
^{1}HNMR ($CDCl_3$): confirms structure assignment.

Elemental Analysis Calc'd for $C_{16}H_{13}N_3O_2$:			
	C, 4.69;	H, 68.81;	N, 15.04.
Found:	C, 4.62;	H, 68.67;	N, 15.08.

EXAMPLE 2

3(R,S)-Amino-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one

A solution of 150 ml of methanol containing 5 g (17.9 mmole) of 1,3-dihydro-1-methyl-3-oximino-5-phenyl-1,4-benzodiazepin-2-one was treated with a slurry of active Raney-nickel catalyst[1] in ethanol (10 g wet weight). The resulting suspension was hydrogenated on a Parr apparatus at 60 psi and 23°C for 30 hours. The catalyst was removed by filtration and the filtrate was concentrated to afford the title compound in 95% yield.
R_f=0.23 (chloroform-ethanol, 95:5), R_f=0.23 (chloroform-methanol-acetic acid-water, 90:10:1:1)
^{1}HNMR ($CDCl_3$): spectrum confirms structure assignment.

EXAMPLE 3

3(S)-(-)-1,3-Dihydro-3-(2-indolecarbonylamino)-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one

3(S)-(-)-3-Amino-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (595 mg, 2.24mmole) was dissolved in CH_2Cl_2 (15 ml) and treated with 2-indolecarbonyl chloride (403 mg, 2.24 mmole) followed by triethylamine (227 mg, 2.24 mmole). The mixture was stirred at room temperature for 30 minutes and concentrated in vacuo. The residue was chromatographed on silica gel (5% Et_2O/CH_2Cl_2) and the combined product fractions evaporated to dryness in vacuo. Three times, Et_2O (15 ml) was added and evaporated in vacuo to give the title compound: (m.p. 168° - 185°C).
TLC: Silica gel (6% Et_2O/CH_2Cl_2), R_f = 0.23
NMR: Consistent with structure
HPLC: Greater than 99% pure.
M.S.: Molecular ion at m/e = 408
$[\alpha]D^{25}$ = -103° (0.0078 g/ml, CH_2Cl_2)

[1] Raney-Nickel catalyst was prepared according to Fieser & Fieser, Reagents for Organic Synthesis, Vol. I, John Wiley & Sons, Inc., New York 1967, p. 729.

Anal. calc'd for $C_{25}H_{20}N_4O_2$:			
	C, 73.51;	H, 4.94;	N, 13.72;
Found:	C, 73.38;	H, 4.80;	N, 13.66.

EXAMPLE 4

3(RS)-(Boc-L-tryptophanyl)amino-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one

3-(RS)-Amino-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (0.1 g, 0.4 mmol), BOC-L-tryptophan (0.12 g, 0.4 mmol), and DCC (0.4 ml of a 1 M solution in CH_2Cl_2, 0.4 mmol) were combined in 2 ml of THF to which were added 2 ml of DMF and 2 ml of CH_2Cl_2. The mixture was treated with triethylamine (0.11 ml), stoppered, and stirred at room temperature for four days. The mixture was treated with citric acid solution (10%, 3 ml) and CH_2Cl_2 (5 ml), shaken and separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 5 ml). The combined organic layers were washed with citric acid (10%, 2 x 5 ml), sodium bicarbonate (10%, 2 x 5 ml), and H_2O (10 ml), dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel (1:1 (v/v) Et_2O/CH_2Cl_2) and the combined product fractions evaporated to dryness in vacuo. The residue was triturated with petroleum ether and the solid dried in vacuo at 70°: (m.p. 173-177°C.

TLC: Single spot (R_f = 0.56, silica gel plate, 10% (v/v) CH_3OH in CH_2Cl_2).

NMR: The spectrum was consistent with the title structure and verified the presence of two diastereomers.

HPLC: Greater than 99.7% pure (36% and 63.7%).

MS (FAB): a molecular ion at m/e = 537.

Anal. calc'd for $C_{31}H_{31}N_5O_4$:			
	C, 69.25;	H, 5.81;	N, 13.03;
Found:	C, 69.48;	H, 6.18;	N, 12.96.

EXAMPLE 5

(R)-N-(2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-(3-methylphenyl)-urea

Equimolar amounts of 3(R)-amino-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one and 3-methylphenylisocyanate were mixed in 8 ml of dry tetrahydrofuran at room temperature. The reaction mixture was allowed to stand for 8 hours and was then filtered. The collected solids were washed with tetrahydrofuran and dried in vacuo over P_2O_5 to give analytical product m.p. 208-210°C.

NMR: Confirms structure assignment of product.

HPLC: Greater than 99% pure.

MS: Molecular ion at m/e=399 (M + H) (FAB).

Anal. Calc'd for $C_{24}H_{22}N_4O_2$:			
	C, 72.34;	H, 5.56;	N, 14.06.
Found:	C, 72.12;	H, 5.84;	N, 14.04.

EXAMPLE 6

3(S)-3-(2-(N-carboxymethylindole)carbonylamino)-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one

Sodium hydride (0.034 g, 0.71 mmole of a 50% dispersion in mineral oil) and 3(S)-(-)-1,3-Dihydro-3-(2-indolecarbonylamino)-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (0.28 g, 0.69 mmole) were combined in dry, degassed DMF (5 ml) and stirred in an ice bath for 40 minutes. Ethyl bromoacetate (0.077 ml, 0.115 g, 0.69 mmole) was added in one portion, and the mixture stirred one hour at room temperature. The DMF was

removed in vacuo, and the residue treated with cold, aqueous sodium bicarbonate solution and extracted with ethyl acetate. The ethyl acetate fractions were combined, washed with water, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted with 7% ether in CH_2Cl_2. The product fractions were combined and evaporated to dryness in vacuo. The residue (0.25 g, 0.53 mmole) was stirred in CH_3OH (5 ml) and treated with aqueous sodium hydroxide (0.7 ml of a 1 N solution; 0.7 mmole). The mixture was stirred overnight at room temperature, then acidified with 1 N HCl and extracted with ethyl acetate. The ethyl acetate fractions were combined, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was crystallized from a mixture of acetone, ether, and petroleum ether to give the title compound: (m.p. 165-195°C (indistinct)).

TLC: Silica gel (90:10:1:1, CH_2Cl_2:CH_3OH:HOAc:H_2O), R_f=0.52
NMR: Consistent with structure
HPLC: Greater than 97% pure
M.S.: Molecular ion at M+H=467 (FAB).

Anal. calc'd for $C_{27}H_{22}N_4O_4 \cdot 0.15\ C_4H_{10}O \cdot 0.45\ H_2O$			
	C, 68.24;	H, 5.06;	N, 11.54;
Found:	C, 68.21;	H, 4.85;	N, 11.47.

EXAMPLE 7

(S)-4-[-2-(((2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)amino)carbonyl)-1H-indolyl-1]-butanoic acid

Sodium hydride (0.1 g, 2.5 mmole of a 60% dispersion in mineral oil) and 3(S)-(-)-1,3-dihydro-3-(2-indolecarbonylamino)-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (1.0 g, 2.45 mmole) were combined in dry, degassed DMF (10 ml) and stirred in an ice bath for 40 minutes. Ethyl-4-bromobutyrate (0.52 g, 2.7 mmole) was added in one portion, and the mixture stirred three hours at room temperature. The DMF was removed in vacuo, and the residue was treated with CH_3OH (350 ml) and aqueous 1 N NaOH (10 ml) and stirred at room temperature for three days. The mixture was evaporated to dryness in vacuo, and the residue was treated with aqueous sodium bicarbonate solution and extracted with ethyl acetate. The aqueous fraction was made acidic with 1N HCl and extracted with ethyl acetate. The ethyl acetate layer was dried over sodium sulfate and evaporated to dryness in vacuo. The residue was chromatographed on silica gel (7% Et_2O/CH_2Cl_2 followed by 540:10:1:1, CH_2Cl_2:
CH_3OH:HOAc:H_2O, and the product fractions evaporated to dryness in vacuo. The residue was crystallized from ether to give the title compound: (m.p. 192-195°C).

TLC: Silica gel (90:10:1:1, CH_2Cl_2:CH_3OH:HOAc:H_2O), R_f=0.23
NMR: Consistent with structure
HPLC: Greater than 97% pure
M.S.: Molecular ion at M+H=495 (FAB)

Anal. calc'd for $C_{29}H_{26}N_4O_4$			
	C, 70.43;	H, 5.30;	N, 11.33;
Found:	C, 70.14;	H, 5.42;	N, 11.36.

EXAMPLE 8

(RS)-1,3-Dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one

3-(RS)-Amino-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (15.1 g, 57 mmole) was dissolved in THF (150 ml), cooled in an ice bath,and treated with triethylamine (7.93 ml). A solution of p-nitrophenylchloroformate (11.45g, 57 mmole) in THF (70 ml) was added dropwise. An additional 1 ml of triethylamine and a solution of 2.0g of p-nitrophenylchloroformate in THF were added. After stirring one hour, the mixture was filtered and evaporated to dryness in vacuo. Ether was added and the mixture stirred one hour at room

temperature and filtered. The solid was washed twice with ether and dried to give the title compound.

EXAMPLE 9

(RS)-3-((((2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)amino)carbonyl)amino)benzoic acid, also known as (RS)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-(3-carboxyphenyl)-urea.

(RS)-1,3-Dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one (5.03 g, 11.2 mmole) and m-aminobenzoic acid (2.4 g, 17.5 mmole) were combined in DMF (120 ml), treated with triethylamine (4.2 ml), and stirred in an oil bath thermostatted at 45° for 18 hours. The DMF was removed in vacuo and the residue was dissolved in boiling methanol. The crystallized product was recrystallized from hot methanol: (m.p. 175-180°C).
TLC: Silica gel (90:10:1:1, CH_2Cl_2:CH_3OH:HOAc:H_2O), R_f=0.5
NMR: Consistent with title structure
HPLC: Greater than 97.8% pure
M.S.: M+H at m/e=429 (FAB)

Anal. calc'd for $C_{24}H_{20}N_4O_4 \cdot 1.15H_2O$			
	C, 64.17;	H, 5.00;	N, 12.47;
Found:	C, 64.20;	H, 5.20;	N, 12.60.

EXAMPLE 10

(R)-3-((((2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)amino)carbonyl)amino)benzoic acid

Benzyl alcohol (10 g, 92.6 mmole) was treated with a solution of m-nitrobenzoyl chloride (17.5 g, 94.5 mmole) in ether (50 ml) added dropwise. The mixture was stirred at room temperature for eighteen hours, then washed twice with aqueous sodium bicarbonate, dried over sodium sulfate, and filtered. The filtrate was evaporated to dryness in vacuo and the residue chromatographed on silica gel eluted with 1:1 CH_2Cl_2:hexane. The product fractions were combined and evaporated to dryness in vacuo. A portion (5.2 g, 20.2 mmole) of the resulting benzyl m-nitrobenzoate was dissolved in ethanol and hydrogenated over platinum oxide (70 mg) at 50 psi of H_2. The resulting mixture was filtered and evaporated to dryness in vacuo to give benzyl m-aminobenzoate.

(R)-1,3-Dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one was prepared using the procedure of Example 8 wherein 3-(R)-Amino-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one was employed in place of the (RS) compound.

To benzyl m-aminobenzoate (0.25 g, 1.10 mmole) in DMF (17 ml) was added triethylamine (0.23 ml) followed by a solution of (R)-1,3-dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one(0.469 g, 1.09 mmole) in DMF (23 ml) containing triethylamine (0.23 ml). The mixture was stirred at room temperature for one hour, then treated with water, made acidic with 1N HCl, and extracted with ethyl acetate. The ethyl acetate layers were combined, washed with aqueous sodium bicarbonate, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted with 500 ml each of 5%, 6%, 7%, 9%, 10%, and 12% ether in CH_2Cl_2. The product fractions were combined and evaporated to dryness in vacuo. A portion of the residue (81.2 mg, 0.086 mmole) was dissolved in ethanol (70 ml) and hydrogenated over palladium/charcoal (20 mg) at 50 psi of H_2. The mixture was filtered and evaporated to dryness in vacuo to provide the title compound.
TLC: Silica gel (90:10:1:1, CH_2Cl_2:CH_3OH:HOAc:H_2O) identical to material prepared as in Example 9.

EXAMPLE 11

3-(RS)-Amino-1,3-dihydro-1-(2-hydroxyethyl)-5-phenyl-2H-1,4-benzodiazepin-2-one

1,3-Dihydro-5-phenyl-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one[1] (0.25 g, 0.65 mmole) was dissolved in DMF (5 ml) stirred in an ice bath. The solution was treated with sodium hydride (32.7 mg, 0.681 mmole of a 50% dispersion in mineral oil) and the mixture stirred for forty minutes in the cold. Oxirane gas was bubbled into the mixture for five minutes, and the resulting mixture heated on a steam bath for one hour. The DMF was removed in vacuo. The residue was treated with water and extracted with ethyl acetate. The ethyl acetate layers were combined, washed with water, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted with 35% ethyl acetate in methylene chloride. The combined product fractions were evaporated to dryness in vacuo. The residue was dissolved in CH_2Cl_2, cooled in an ice bath, and saturated with HBr gas. The mixture was evaporated to dryness in vacuo, treated with a minimum volume of water and extracted repeatedly with ethyl acetate. The ethyl acetate layers were combined, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo to give the title compound.

EXAMPLE 12

(RS)-N-(2,3-Dihydro-1-(2-hydroxyethyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-1H-indole-2-carboxamide

3-(RS)-Amino-1,3-dihydro-1-(2-hydroxyethyl)-5-phenyl-2H-1,4-benzodiazepin-2-one (69.0 mg, 0.234 mmole), indole-2-carbonyl chloride (43.1 mg, 0.240 mmole) and triethylamine (33.3 μl, 0.240 mmole) were combined in CH_2Cl_2 (3 ml). The reaction was stirred for 10 minutes at room temperature then chromatographed on silica gel (14% acetone in CH_2Cl_2). The product fractions were combined and evaporated to dryness in vacuo. The residue was triturated with Et_2O to yield the title compound: (m.p. 160-171°C).
TLC: silica gel (15% acetone in CH_2Cl_2) R_f= 0.27
NMR: Consistent with structure
HPLC: 97.6% M.S.: Molecular ion at m/e=438

Anal. Calc'd for $C_{26}H_{22}N_4O_3 \cdot 0.1C_4H_{10}O \cdot 0.25H_2O$			
	C, 70.40;	H, 5.26;	N, 12.44
Found:	C, 70.40;	H, 5.16;	N, 12.15

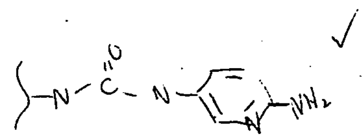
EXAMPLE 13

(RS)-N-(2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-9H-pyrido(3.4-b)indol-3-yl-urea

A solution of (RS)-1,3-dihydro-1-methyl-3-(p-(-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one (100 mg, 0.232 mmole) and 3-amino-β-carboline[1] (45.8 mg, 0.250 mmole) in DMF (5 ml) was treated with triethylamine (48.4 μl, 0.348 mmole) and warmed to 45°C for 16 hours. After removal of DMF in vacuo, the residue was dissolved in CH_2Cl_2 and chromatographed on silica gel (25% acetone in CH_2Cl_2). The product fractions were combined and stripped and the title compound crystallized from EtOAc: (m.p. 281-283°C).
TLC: silica gel (160/10/1 of CH_2Cl_2/MeOH/conc. NH_4OH) R_f= 0.24
NMR: Consistent with structure
HPLC: 99.3% pure
M.S.: M+H= 475 (FAB)

[1]Bock, M.G., et al., J. Org. Chem., 52, 3232 (1987).
[1] Dodd, R. H., et al. J. Med. Chem. 28 824 (1985)

Anal. Calc'd for $C_{28}H_{22}N_6O_2 \cdot 0.20C_4H_8O_2$			
	C, 70.28;	H, 4.83;	N, 17.08
Found:	C, 70.10;	H, 4.55;	N, 17.24

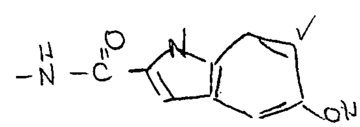


EXAMPLE 14

(RS)-N-(6-Amino-3-pyridyl)-N'-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-urea

2,5-Diaminopyridine dihydrochloride (45.5 mg, 0.250 mmole), (RS)-1,3-dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one (100 mg, 0.232 mmole) and triethylamine (110 µl, 0.79 mmole) were combined in DMF (8 ml) and stirred at room temperature for 16 hours. After removal of DMF in vacuo, the residue was treated with 1N NaOH (aqueous) and extracted with EtOAc (3x). The organic layers were combined, washed with brine (1x), dried over Na_2SO_4, filtered and evaporated to dryness in vacuo. The crude residue was chromatographed on silica gel eluted with 7% MeOH in CH_2Cl_2. The product fractions were combined and evaporated to dryness in vacuo. The residue was crystallized from EtOAc diluted with Et_2O to give the title compound: (m.p. 165-175°C).
TLC: silica GF (90/10/1/1 of $CH_2Cl_2/MeOH/H_2O/HOAc$) R_f= 0.22
NMR: consistent with structure
HPLC: 96.3%
M.S.: M+H= 401 (FAB)

Anal. Calc'd for $C_{22}H_{20}N_6O_2 \cdot 0.35H_2O$			
	C, 64.96;	H, 5.13;	N, 20.66
Found:	C, 65.05;	H, 5.20;	N, 20.66.



EXAMPLE 15

1,3-Dihydro-3-(5-hydroxyindole-2-carbonylamino)-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one

3(S)-(-)-3-Amino-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (0.14 g, 0.53 mmol) and 5-hydroxyindole-2-carboxylic acid (0.11 g, 0.63 mmol) were combined in a mixture of CH_2Cl_2 (5 ml) and DMF (1 ml). EDC (0.1 g, 0.56 mmol) was added followed by Et_3N sufficient to render the mixture basic (pH 8) to moistened pH detector sticks (E. Merck). The mixture was stirred at ambient temperature for 6 hours, then evaporated to dryness in vacuo. The residue was diluted with aqueous citric acid and extracted with EtOAc. The EtOAc layer was washed twice with saturated sodium bicarbonate which had been diluted 1:1 with water, then dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was dried in vacuo at 90°C overnight to give the title compound: (mp 120-130°C (↑)).
TLC: Silica gel (10% CH_3OH in CH_2Cl_2) Rf = 0.73
NMR: Consistent with structure, H_2O observed.
HPLC: Greater than 94.5% pure
M.S. Molecular ion at m/e = 424

Anal. Calc'd for $C_{25}H_{20}N_4O_3 \cdot 0.55H_2O$:			
	C, 69.12;	H, 4.90;	N, 12.90;
Found:	C, 69.34;	H, 5.01;	N, 12.52.

EXAMPLE 16

1,3-Dihydro-3-(5-carboxymethyloxyindole-2-carbonylamino)-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one

1,3-Dihydro-3-(5-hydroxyindole-2-carbonylamino)-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (0.1

g, 0.236 mmol) and iodoacetic acid (0.044 g, 0.236 mmol) were combined in dry DMF (2 ml) and treated with sodium hydride (18.8 mg of a 60% suspension in mineral oil; 0.472 mmol). The mixture was stirred at ambient temperature for 1 hour, then evaporated to dryness in vacuo. To the residue were added water, dilute sodium bisulfite solution, then saturated sodium bicarbonate. The aqueous phase was washed with EtOAc, made acidic with 6N HCl, and extracted with EtOAc. The acid layer extract was dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted wth 180:10:1:1 of CH_2Cl_2:MeOH:HOAc:H_2O. The product fractions were evaporated to dryness in vacuo and the residue triturated with ether to give the title compound which was dried in vacuo at 90°C overnight (mp 150-180°C (↑)).
TLC: Silica gel (180:10:1:1 of CH_2Cl_2:CH_3OH:HOAc:H_2O) R_f = 0.19
NMR: Consistent with structure, Et_2O and H_2O observed.
HPLC: Greater than 83.2% pure
M.S. M + H at m/e = 483 (FAB)

Anal. Calc'd for $C_{27}H_{22}N_4O_6 \cdot 0.05Et_2O \cdot 0.7H_2O$:			
	C, 65.49;	H, 4.83;	N, 11.23;
Found:	C, 65.53;	H, 4.49;	N, 11.10.

EXAMPLE 17

N-(2,3-Dihydro-1-(2-hydroxyethyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-(3-methylphenyl)-urea

3-(RS)-Amino-1,3-dihydro-1-(2-hydroxyethyl-5-phenyl-2H-1,4-benzodiazepin-2-one (0.45 g, 1.5 mmol) was dissolved in THF (10 ml) and treated with 3-methylphenylisocyanate (0.207 g, 1.55 mmol), and the mixture stirred at ambient temperature for 1 hour, then evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted with 20% acetone in CH_2Cl_2. The product fractions were evaporated to dryness in vacuo and the residue triturated with ether to give the title compound which was dried in vacuo at 65°C for 2 hours: (mp 138-154°C).
TLC: Silica gel (90:4:0.4:0.4 of CH_2Cl_2:CH_3OH:HOAc:H_2O) R_f = 0.24
NMR: Consistent with structure.
HPLC: Greater than 99.7% pure
M.S. M + H at m/e = 429 (FAB)

Anal. Calc'd for $C_{25}H_{24}N_4O_3 \cdot 0.07\ Et_2O \cdot 0.4\ H_2O$:			
	C, 68.87;	H, 5.83;	N, 12.71;
Found:	C, 68.83;	H, 5.63;	N, 12.58.

EXAMPLE 18

N-(2,3-Dihydro-1-(2-dimethylaminoethyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-(3-methoxyphenyl)-urea

Sodium hydride (26.4mg of a 50% dispersion in mineral oil; 0.55mmol) was stirred under nitrogen in dry DMF (5ml) in an ice bath. (RS)-1,3-Dihydro-3-(benzyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one (0.21g, 0.54mmol) in DMF (4ml) was added, and the mixture stirred 1hr in the cold. (2-Chloroethyl)-dimethylamine (59.2mg, 0.55mmol), prepared by distillation of a mixture of the hydrochloride and powdered sodium hydroxide in vacuo, was added and the mixture stirred 1hr in the cold, and overnight at ambient temperature. The DMF was removed in vacuo and the residue was treated with water and extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over sodium sulfate, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted with 90:10:1:1 of CH_2Cl_2:MeOH:H_2O:HOAc and the product fractons were evaporated to dryness in vacuo to provide (RS)-1-(2-chloroethyl)-1,3-dihydro-3-(benzyloxycarbonyl) amino-5-phenyl-2H-1,4-benzodiazepin-2-one. This compound (120mg, 0.268mmol) was added to a suspension of 10% palladium/Carbon (70mg) in 4.5% methanolic formic acid (5ml) stirred at ambient temperature under nitrogen. After 25 min, the mixture was filtered and the filtrate evaporated to dryness in va-

cuo. The residue was treated with saturated sodium carbonate solution and extracted with ethyl acetate. The combine ethyl acetate layers were washed with water, dried over sodium-sulfate, filtered, and evaporated to dryness in vacuo. The residue was dissolved in THF, cooled in an ice bath, and treated with 3-methoxyphenylisocyanate (35.1 µl). The mixture was stirred in the cold for 30 min. warmed to ambient temperature, and filtered. The filtrate was evaporated to dryness in vacuo, and the residue was treated with ether (30ml) and re-evaporated three times. The residue was triturated with ether and filtered, and the resulting solid dried at 65°C overnight to provide the title compound: (mp 213-215°C).TLC: Silica gel (80:10:1 of CH_2Cl_2:CH_3OH:NH_3) R_f=0.41

NMR: Consistent with structure.
HPLC: Greater than 99.3% pure
M.S. M+H at m/e = 472 (FAB)

Anal. calc'd for $C_{27}H_{29}N_5O_3$:			
	C, 68.77;	H, 6.20;	N, 14.85;
Found:	C, 68.43;	H, 6.30;	N, 14.75.

EXAMPLE 19

(R)-3-((((2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)amino)carbonyl)amino)benzoic acid ethyl ester

(R)-1,3-Dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one (150mg, 0.35mmol), 3-aminobenzoic acid ethyl ester (61mg, 0.37mmol), and triethylamine (52.5 mg, 0.52mmol) were combined in DMF (2ml) and heated at 45° overnight. The DMF was removed in vacuo and the residue crystallized from ethyl acetate to provide the title compound: (mp 140-142°C).

TLC: Silica gel (1:1 ethyl acetate:hexane) R_f=0.27
NMR: Consistent with structure.
HPLC: Greater than 96.6% pure
M.S. Molecular ion at m/e = 456

Anal. calc'd for $C_{26}H_{24}N_4O_4{\cdot}0.5H_2O$:			
	C, 67.09;	H, 5.41;	N, 12.04;
Found:	C, 67.09;	H, 5.25;	N, 11.87.

EXAMPLE 20

(R)-3-((((2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)amino)carbonyl)amino)phenylacetic acid

(R)-1,3-Dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one (1.92g, 4.47mmol) was dissolved in THF (25ml) and treated with a solution of (3-aminophenyl)acetic acid methyl ester (670mg, 4.06mmol) in THF (5ml) followed by triethylamine (615mg, 6.09mmol). The mixture was stirred at ambient temperature for 4 days. The solvent was removed in vacuo and the residue was treated with water (20ml) and extracted with ethyl acetate. The combined ethyl acetate layers were washed with 1M NaOH, then with 10% citric acid, dried over sodium sulfate, filtered and evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted with 1:1 hexane:ethyl acetate. The product fractions were combined and evaporated to dryness in vacuo and the residue crystallized from ethyl acetate to give (R)-3-((((2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)amino)carbonyl)amino)phenyl acetic acid methyl ester. This ester (885mg, 1.94mmol) was dissolved in THF (5ml) and treated with a solution of lithium hydroxide (815mg, 19.4mmol) in water (10ml). The mixture was stirred at ambient temperature for 3 hours, diluted with water (100ml), acidified with 1N HCl, and extracted with ethyl acetate. The ethyl acetate layers were washed with brine, dried over sodium sulfate, filterate, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel eluted with chloroform followed by 9:1 chloroform:methanol. The product fractions were

combined and evaporated to dryness in vacuo. The residue was crystallized from ethyl acetate to provide the title compound: (mp 167-170°C).
TLC: Silica gel (1:1 ethyl acetate:hexane) single component.
NMR: Consistent with structure.
HPLC: Greater than 97% pure.
M.S. M+H at m/e=443(FAB).

Anal. Calc'd for $C_{25}H_{22}N_4O_4{\cdot}0.55EtOAc$:			
	C, 66.54;	H. 5.42;	N, 11.41;
Found:	C, 66.15;	H, 5.04;	N, 11.63.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations or modifications, as come within the scope of the following claims and its equivalents.

EXAMPLE 21

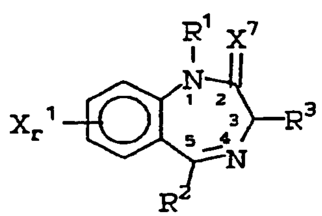
(R)-N-(2,3-Dihydro-1H-inden-5-yl)N'-(2,3 dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-urea

(R)-1,3-Dihydro-1-methyl-3-(p-nitrophenyloxycarbonyl)amino-5-phenyl-2H-1,4-benzodiazepin-2-one (200 mg. 0.46 mmole) was dissolved in 2 ml of freshly degassed, dry N,N-dimethylformamide (DMF) and treated with 1 ml of DMF containing 74 mg (0.50 mmole) of 5-aminoindane and 97.4 μl of triethylamine. The resulting solution was stirred under nitrogen for four hours. The reaction mixture was poured into 75 ml of water and extracted with ethyl acetate (3 X 40 mL). The combined organic extracts were washed with 1 N sodium hydroxide solution (4 X 100 mL), 10% citric acid solution (2 X 100 mL), and brine. The organic extracts were then dried and the residue was plug-filtered through a six inch silica gel column. The eluate was concentrated and the residue was crystallized from a methylene chloride-ether mixture to give the title compound: mp 156-158°C
NMR:
Structure is consistent with the spectrum.
FAB MS: 425 (M^++H).

Anal. Calc'd for $C_{26}H_{24}N_4O_2$:			
Calc'd:	C, 73.56;	H, 5.69;	N, 13.20.
Found:	C, 73.26;	H, 5.81;	N, 13.04.

Claims

1. A pharmaceutical composition useful in the treatment of oncologic disorders, controlling pupil constriction in the eye, or treating a withdrawal response produced by chronic treatment or abuse of drugs or alcohol, comprising a therapeutically effective amount of a compound of the formula:



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wherein:

R^1 is H, C_1-C_6 linear or branched alkyl, loweralkenyl, lower alkynyl, $X^{12}COOH$, $-X^{12}COOR^6$, $-X^{11}$-cycloloweralkyl, $-X^{12}NR^4R^5$, $-X^{12}CONR^4R^5$, $-X^{12}CN$, or $X^{11}CX_3{}^{10}$;

R^2 is H, loweralkyl, substituted or unsubstituted phenyl (wherein the substitutents may be 1 or 2 of halo, loweralkyl, loweralkoxy, loweralkythio, carboxyl, carboxyloweralkyl, nitro, $-CF_3$, or hydroxy), 2-,3-, or 4-pyridyl;

R^3 is

$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X^{11}R^{7},$$

$-NH(CH_2)_{2-3}NHCOR^7$,

$$-X^{11}\overset{O}{\overset{\|}{C}}X^{9}X^{11}R^{7},$$

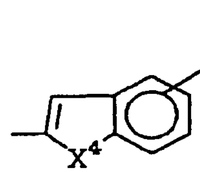
$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X_a{}^{9}X^{11}R^{7},$$

or $-X^{11}NR^{18}SO_2(CH_2)_qR^7$;

R^4 and R^5 are independently H or R^6 or in combination with the N of the NR^4R^5 group form an unsubstituted or mono or disubstituted, saturated or unsaturated, 4-7 membered heterocyclic ring, or benzofused, 4-7 membered heterocyclic ring wherein said heterocyclic ring or said benzofused heterocyclic ring may contain a second heteroatom selected from O and NCH_3 and the substituent(s) is/are independently selected from C_1-C_4alkyl;

R^6 is loweralkyl, cycloloweralkyl, substituted or unsubstituted phenyl, or substituted or unsubstituted phenylloweralkyl wherein the phenyl or phenylloweralkyl substituents may be 1 or 2 of halo, loweralkyl, loweralkoxy, nitro, or CF_3;

R^7 is



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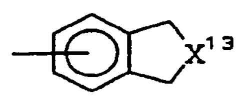

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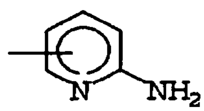

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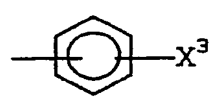

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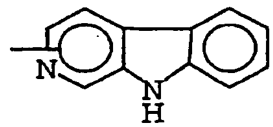
 ;

R^8 is H, loweralkyl, cycloloweralkyl, $-X^{13}CONH_2$, $-X^{13}COOR^6$, $-X^{13}COOH$, X^{13}-cycloloweralkyl, or $-X^{13}NR^4R^5$,

R^{15} is H, loweralkyl, or cycloloweralkyl;

R^{18} is H or loweralkyl;

n is 1-6,

q is 0-4;

r is 1 or 2;

X^1 is H,$-NO_2$,CF_3, CN, OH, loweralkyl, halo, loweralkylthio, loweralkoxy, $-X^{11}COOR^8$, $X^{11}COOH$, or $-X^{11}NR^4R^5$,

X^2 is H or X^3, with the proviso that when X^2 is H, then X^4 is NX^5COOH or NX^5COOR^6 wherein X^5 is a linear alkyl chain of 2 to 6 carbon atoms, any carbon atom of which may be additionally substituted with a linear or branched alkyl group of 1 to 3 carbon atoms;

X^3 is $O(CH_2)_nCOOR^6$, $O(CH_2)_nCOOH$, $(CH_2)_nCOOR^6$, $(CH_2)_nCOOH$, $COOR^6$, or $X^{12}OR^6$;

X^4 is S,O, CH_2, or NR^8;

X^7 is O,S, HH, or $N(R^{15})_2$ with the proviso that X^7 can be $N(R^{15})_2$ only when R^1 is not H;

X^8 is H, loweralkyl;

X^9 and X_a^9 are independently NR^{18} or O;

X^{10} is F, Cl, or Br;

X^{11} is absent or C_1-$_4$ linear or branched alkyl;

X^{12} is C_{1-4} linear or branched alkylidene,

X^{13} is C_{1-4} linear or branched alkyl;

or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition useful in the treatment of oncologic disorders, controlling pupil constriction in the eye, or treating a withdrawal response produced by chronic treatment or abuse of drugs or alcohol, comprising a therapeutically effective amount of a compound of Claim 1, wherein:

R^1 is H, C_1-C_6 linear or branched alkyl, $-X^{12}COOR^6$, $-X^{11}$-cycloloweralkyl, $X^{12}NR^4R^5$, $-X^{12}CONR^4R^5$, or $X^{12}COOH$;

R^2 is substituted or unsubstituted phenyl (wherein the substitutents may be 1 or 2 of halo, loweralkyl, loweralkoxy, loweralkylthio, carboxyl, carboxyloweralkyl, nitro, $-CF_3$ or hydroxy), 2-,3-, or 4-pyridyl;

R^3 is

$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X^{11}R^7,$$

$$-X^{11}\overset{O}{\overset{\|}{C}}X^9X^{11}R^7,$$

$-NH(CH_2)_{2-3}NHCOR^7$, or

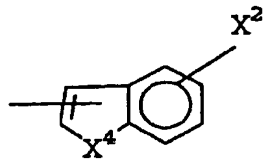
$$-X^{11}NR^{18}\overset{O}{\overset{\|}{C}}X^9X^{11}R^7;$$

R^4 and R^5 are independently H or R^6 or in combination with the N or the NR^4R^5 group form an unsubstituted or mono or disubstituted, saturated or unsaturated, 4-7 membered heterocyclic ring, or benzofused 4-7 membered heterocyclic ring wherein said heterocyclic ring or said benzofused heterocyclic ring may contain a second heteroatom selected from O and NCH_3 and the substituent(s) is/are indepen-

dently selected from C_1-C_4 alkyl;

R^6 is C_{1-4} straight or branched-chain alkyl or C_3-C_6 cycloalkyl

R^7 is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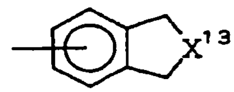
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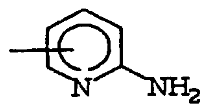
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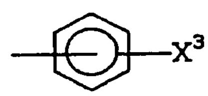
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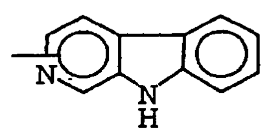
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R^8 is H, loweralkyl, cycloloweralkyl, $X^{13}COOR^6$, $X^{13}COOH$, or $X^{13}NR^4R^5$;

R^{18} is H or loweralkyl;

n is 1-3;

q is 0-3;

r is 1 or 2;

X^1 is H, $-NO_2$, CF_3, CN, loweralkyl, halo, loweralkylthio $-X^{11}COOR^6$, $X^{11}COOH$, or $X^{11}NR^4R^5$;

X^2 is H or X^3 with the proviso that when X^2 is H, then X^4 is NX^5COOH or NX^5COOR^6 wherein X^5 is a linear alkyl chain of 2 to 4 carbon atoms, any carbon atom of which may be additionally substituted with a linear or branched alkyl group of 1 to 3 carbon atoms;

X^3 is $O(CH_2)_nCOOR^6$, $O(CH_2)_nCOOH$, $(CH_2)_nCOOR^6$, $(CH_2)_nCOOH$, or $COOR^6$;

X^4 is S, O, or NR^8;

X^7 is O;

X^9 and X_a^9 are independently NR^{18}, or O;

X^{11} is absent or C_{1-4} linear alkyl;

X^{12} is C_{1-4} linear or branched alkylidene;

X^{13} is C_{1-4} linear or branched alkyl;

or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

3. A pharmaceutical composition according to Claim 1, wherein the therapeutically effective amount of the compound of Formula I is from about 0.005 mg/kg to about 50 mg/kg of body weight.

4. The use of a compound of Claim 1 for the manufacture of a medicament for treating oncologic disorders, controlling pupil constriction in the eye, or treating a withdrawal response produced by chronic treatment or abuse of drugs or alcohol.

5. The use according to Claim 4, wherein the compound of Formula I is present in the medicament manufactured in an amount of from about 0.005 mg/kg to about 50 mg/kg of body weight, administered in a single or divided dose.